supernatant fluid collected between 3rd and 5th days post infection of primary cultures of RIF-free chick embryo fibroblasts[6] inoculated with a multiplicity of 100 myeloblast AMV particles per cell. Supernatants so obtained contained around 3×10^9 virus particles per ml. Both types of viruses were purified in a similar way by 4 alternate low and high speed centrifugations[7]. The purified viral pellets were treated with TE as described by ECKERT et al.[8] prior to use as immunogens or test antigens. All preparations of disrupted virus were exhaustively dialyzed against phosphate buffered saline (pH 7.4).

Rabbits were given an initial inoculation of detergent treated myeloblast virus of 10^{12} particles emulsified either in Freund's incomplete or complete adjuvent. Booster injections were given 5 weeks following primary immunization and consisted of 5×10^{11} disrupted particles prepared in a similar way. All inoculations were in the hind foot pads. The rabbits were bled from the ear 4 weeks following primary immunization and 10 days following booster injections. Serum was stored at -20 °C.

Gels were prepared with 1% noble agar on microscopic slides. 10 µl of antigen preparations were added into the slots and electrophoresis carried out with 0.1 *M Tris* buffer at pH 8.0 for 50 min. Following the electrophoresis a trough was cut in the middle of the slide and 100 µl of the antiserum was added. The slides were incubated at 37 °C for 24–72 h before reading. They were then washed for 1–2 days and stained with amido black. The stained preparations were then photographed.

The Figure shows precipitin lines obtained with both virus preparations when reacted against the rabbit antiserum. While myeloblast AMV split products gave 4 lines of reaction, the fibroblast virus showed only 2 lines. These 2 precipitin lines present in the fibroblast virus, however, show identity of reaction with the corresponding lines of myeloblast virus. The remaining 2 lines of myeloblast virus, of which one is prominent and migrates towards cathode, and another faint one are absent in the fibroblast virus split products.

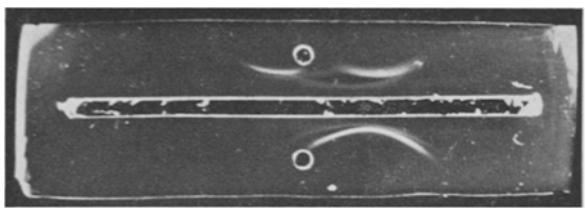
Earlier studies have used complement fixation (CF) to determine the number of antigens present in the AMV[8]. Since CF test is limited in its ability to distinguish the number of antigens contributing to a positive reaction, we have employed immunoelectrophoresis to demonstrate precipitating antigens contained with in the disrupted virus preparations. In a similar study but using avian tumor group specific antisera from Rous sarcoma tumour bearing hamsters, it was shown that SDS treated AMV contains 3 antigenic components[9]. Our studies confirm that multiple antigenic components are present in AMV.

In a comparative study of the viral antigens present in the AMV infected myeloblast and fibroblasts, it was shown that chick tissue components of the virus present in the myeloblast cell are absent in the fibroblast cell[10]. A similar difference is now indicated by the AMV produced by these 2 different host cells. These two studies and those already mentioned clearly demonstrate that AMV incorporates cellular material, as it buds from the surface of a host cell, and in this respect AMV produced by different host cells shows variation in its constituent components of cellular origin.

Zusammenfassung. Immunelektrophoretisch wird ein verschiedenes Verhalten des Vogel-Myeblastosis-Virus nachgewiesen, je nachdem, ob dieses in Myeloblasten oder in Fibroblasten gezüchtet wurde.

P. R. RAO[11]

Department of Zoology,
Osmania University Post-Graduate Centre,
Warangal-1 (A. P., India), 8 March 1972.



Immunoelectrophoresis of TE split AMV from myeloblast (upper well) and fibroblast (lover well) cells. The trough contained rabbit antiserum of AMV split products. Precipitin lines migrating towards cathode present in the myeloblast AMV are absent in the fibroblast AMV.

[6] H. RUBIN, Proc. natn. Acad. Sci., USA *46*, 1105 (1960).
[7] R. A. BONAR and J. W. BEARD, J. natn. Cancer Inst. *23*, 183 (1959).
[8] E. A. ECKERT, R. ROTT and W. SCHAFER, Virology *24*, 426 (1964).
[9] G. ROTH and R. M. DOUGHERTY, Virology *40*, 1030 (1970).
[10] P. R. RAO, CURR. Sci. *40*, 81 (1971).
[11] This work was done while the author was at Duke University Medical Centre, Durham (N.C., USA).

Isolation and some Properties of the Heparin-Neutralizing Factor (PF 4) Released from Human Blood Platelets

A heparin-neutralizing activity in extracts from blood platelets, subsequently termed platelet factor 4 (PF 4), has been described as early as 1951 by VAN CREVELD and PAULSSEN[1]. First attempts at characterizing this material were made by DEUTSCH et al.[2,3], who finally obtained by chromatography on DEAE-Sephadex A-50 a 950-times enriched product. These and other studies, in particular by POPLAWSKI and NIEWIAROWSKI[4], as well as by FARBISZEWSKI et al.[5] led to the assumption that PF 4 is a protein of relatively low molecular weight or a large polypeptide[6]. In recent years it has been found that PF 4 may interact with fibrinogen and certain fibrinogen-complexes and in this form may contribute to platelet aggregation[7,8] and play a role in intravascular coagulation[9]. Therefore a more detailed study of some of the properties of purified PF 4 seemed justified.

Since PF 4 is released by thrombin from platelets in a fast reaction[10] it seemed appropriate to use the supernatant from thrombin-treated platelets as the starting material for its isolation, the more as the specific activity of the released material as calculated on a protein basis is up to 20 times higher than that of homogenized platelets.

Platelet factor 4 activity was determined according to a slight modification of the method by HARADA and ZUCKER[11]. Platelets were isolated from the buffy coats of

ACD-blood collected for the Central Laboratory of the Blood Transfusion Service of the Swiss Red Cross as previously described[12]. They were washed twice by centrifugation as described by MASSINI and LÜSCHER[13] and once more in saline buffered to pH 7.4 with *Tris*-maleate (8 vol. saline, 2 vol. 0.05 *M* *Tris*-maleate buffer pH 7.4). The final suspension medium consisted of the same solution, but contained additionally 1.5 m*M* $MgCl_2$, 5 m*M* KCl, 2.5 m*M* $CaCl_2$ and 2.8 m*M* glucose.

Isolation of PF 4. The concentrated suspension (10^{10} platelets/ml) was stirred slowly at 37 °C; thrombin (Hoffmann-La Roche, Basel) was added to a final concentration of 1 U/ml and stirring was continued for 3 min. After centrifugation at 4500 *g* for 10 min, the supernatant was brought to 56 °C for 10 min (cf. 6); it was then cooled and dialyzed in the cold against 0.005 *M* phosphate buffer pH 7.0 until essentially free of other ions. After centrifugation at 100000 × *g* for 45 min the supernatant was brought to pH 5.4 with 0.125 *M* acetate buffer, pH 4.9. A euglobulin precipitate formed which was dissolved in buffered saline (8 vol. 0.15 *M* NaCl, 2 vol. 0.15 *M* *Tris*-HCl, pH 7.4).

Gel filtration of PF 4. The euglobulin-containing solution should contain about 2% protein; it was brought on a Sephadex G-200 column (2 × 95 cm) and eluted with the same *tris*-saline buffer. All the activity was found in the first peak. This shows the active component to be a material of rather high molecular weight. Therefore, the following separations were performed on Biogel A-5 m, 100–200 mesh (Bio-Rad, Richmond, California). In a medium containing *Tris* buffered saline (pH 7.4) 2 peaks were eluted, whereby the PF4-activity was mainly

[1] S. VAN CREVELD and M. M. PAULSSEN, Lancet *2*, 2242 (1951).
[2] E. DEUTSCH and W. KAIN in *Blood Platelets*, Henry Ford Hospital Symp. (Eds. S. A. JOHNSON, R. W. MONTO, J. W. REBUCK and R. C. HORN; Little Brown, Boston 1961), p. 337.
[3] E. DEUTSCH and K. LECHNER, in *Biochemistry of Blood Platelets* (Eds. E. KOWALSKI and S. NIEWIAROWSKI, Academic Press, London and New York 1967), p. 23.
[4] A. POPLAWSKI and S. NIEWIAROWSKI, Biochim. biophys. Acta, *90*, 403 (1964).
[5] R. FARBISZEWSKI, S. NIEWIAROWSKI and A. POPLAWSKI, Biochim. biophys. Acta *115*, 397 (1966).
[6] S. NIEWIAROWSKI, R. FARBISZEWSKI and A. POPLAWSKI, in *Biochemistry of Blood Platelets* (Eds. E. KOWALSKI and S. NIEWIAROWSKI; Academic Press, London and New York 1967) p. 35.
[7] S. NIEWIAROWSKI, R. FARBISZEWSKI, A. POPLAWSKI and B. LIPINSKI, in *Stoffwechsel und Membranpermeabilität von Erythrocyten und Thrombocyten* (Eds. E. DEUTSCH, E. GERLACH und K. MOSER; Georg Thieme, Stuttgart 1968), p. 314.
[8] J. R. O'BRIEN, Ser. Haemat. *3*, 68 (1970).
[9] Z. WEGRZYNOWICZ, M. KOPEC and Z. S. LATALLO, Scand. J. HAEMAT., Suppl. *13*, 49 (1971).
[10] S. NIEWIAROWSKI and D. P. THOMAS, Nature, Lond. *222*, 1269 (1969).
[11] K. HARADA and M. B. ZUCKER, Thromb. Diath. haemorrh. *25*, 41 (1971).
[12] M. BETTEX-GALLAND and E. F. LÜSCHER, Thromb. Diath. haemorrh. *4*, 178 (1960).
[13] P. MASSINI and E. F. LÜSCHER, Thromb. Diath. haemorrh. *25*, 13 (1971).

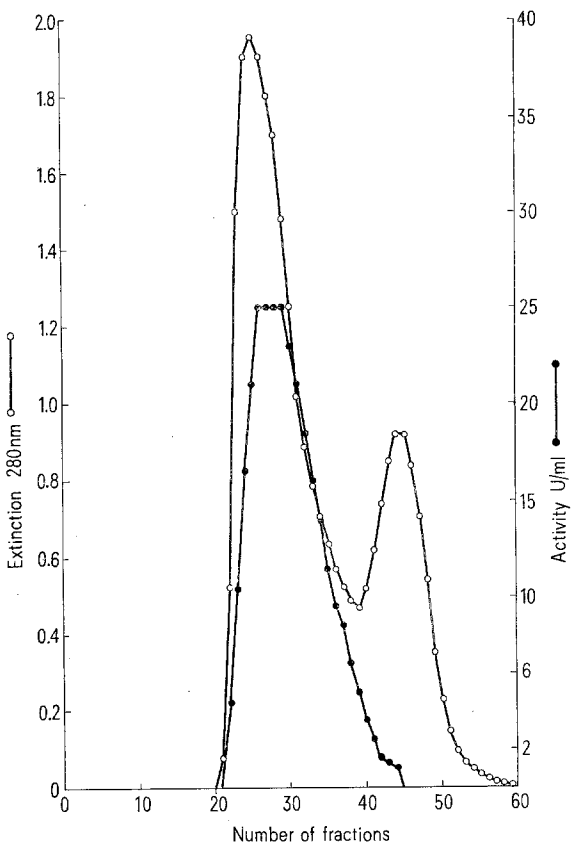


Fig. 1. Gelfiltration of euglobulin fraction of material released from platelets by thrombin on Biogel A-5 m at ionic strength 0.15 μ. Absorption at 280 nm (empty circles) and heparin-neutralizing activity (full circles) are plotted against volume of effluent.

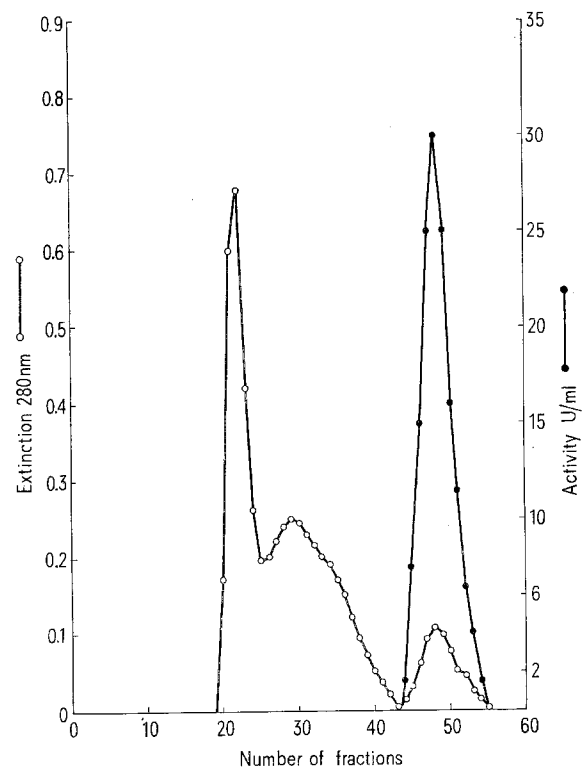


Fig. 2. Gelfiltration at ionic strength 0.75 μ of pooled, heparin-neutralizing fractions obtained in experiment shown in Figure 1. Empty circles, absorbency at 280 nm; full circles; heparin-neutralizing activity.

localized in the descending branch of the first, high-molecular peak (Figure 1).

The pooled active fractions were again submitted to gelfiltration on a comparable column, but at an ionic strength of 0.75 (*tris* buffered saline containing additional 0.6 *M* NaCl). This time, instead of 2, 3 peaks were obtained. The entire PF 4-activity was found in the 3rd peak (Figure 2). The higher salt concentration thus leads to a dissociation of a complex consisting of relatively low-molecular PF 4 and a high molecular carrier-material, to which the activity is bound at a physiological ionic strength.

The active fractions concentrated to a protein content (determined by the biuret method) of 0.6% neutralized 50 to 60 U of heparin per mg protein, as compared to 0.4 U/mg of homogenized platelets or 5–8 U/mg supernatant protein released by thrombin. The purification factors, based on a protein basis, thus range from 150 to about 10, depending upon the starting material. The difference to the much higher values given by DEUTSCH et al.[3] remains unexplained. Below an ionic strength of 0.3, purified PF 4 is only poorly soluble. It migrates, however, in disk electrophoresis at pH 2.9; a single band is thereby formed. In the ultracentrifuge (Spinco model E), a single peak with a sedimentation constant $S_{20,w}$ of 2.6 is observed. The diffusion constant was found to be 8.5×10^{-7}. Assuming a partial specific volume of 0.725, a molecular weight of 29,000 can be calculated.

From peak 2 eluted from the biogel-column at high ionic strength (Figure 2), a material was obtained after removal of contaminant protein by precipitation at 15% (w/v) Na_2SO_4 which was uniform in the ultracentrifuge ($S_{20,w} = 2.8$); the diffusion coefficient was 2.58×10^{-7}. This material which is rich in carbohydrate appears to be the carrier of PF 4 at physiological ionic strength. This was proven by recombination at 0.15 μ: a product which, on ultracentrifugation showed a single component was obtained on saturation of the carrier with PF 4; this recombined material is perfectly soluble and sediments with $S_{20,w} = 12.1$; the diffusion constant is 2.85×10^{-7}. It should be noted that this fully saturated material is not identical with the released complex, which sediments much slower.

These experiments show that PF 4 is released from platelets by thrombin in the form of a complex of high molecular weight. At high ionic strength this complex dissociates into a carrier material and PF 4, the latter of a molecular weight of about 30,000. PF 4 thus obtained is poorly soluble below an ionic strength of 0.3 μ; the carrier was also isolated and recombines with PF 4 at physiological salt concentration to a perfectly soluble complex.

Zusammenfassung. Der in Form eines hochmolekularen Komplexes von den Plättchen freigesetzte, Heparin-neutralisierende Faktor (PF 4) konnte reversibel in ein Träger-Material und ein aktives Protein (Molekulargewicht ca. 30000) aufgespalten werden.

ROSMARIE KÄSER-GLANZMANN, MILICA JAKÁBOVÁ and E. F. LÜSCHER[14]

Theodor-Kocher-Institute, University of Berne, Freiestrasse 1, CH-3000 Bern (Switzerland), 2 May 1972.

[14] This work was supported by the 'Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung'.

Modifications du nombre d'unités formatrices de colonies spléniques par des bactéries induisant l'immunostimulation non spécifique

L'introduction dans l'organisme de facteurs bactériens d'immunostimulation provoque de profondes modifications, telles l'hypertrophie hépato-splénique, l'augmentation non spécifique des immunoglobulines sériques[1], parfois une lymphocytose[2] ou la stimulation des fonctions phagocytaires et lytiques du macrophage[3, 4]. Les modifications du nombre d'unités formant des colonies spleniques (UFC) peuvent être considérées comme l'un de ces effets liés à l'introduction de l'adjuvant systémique. Ayant précédemment posé l'hypothèse d'une corrélation entre l'activité adjuvante de *Brucella* et l'enrichissement de l'organisme en cellules souches[5], nous avons été amenés à étudier plus à fond ce phénomène.

Des souris femelles (C_{57} $Bl_6 \times DBA_2$) F_1, âgées de 6 semaines ont reçu par voie intraveineuse différentes suspensions bactériennes connues pour leurs propriétés immunostimulantes: *Brucella abortus*, souche B_{19} inactivée (B_{19}): 0,5 mg de poids sec par souris (aimablement fourni par le Professeur PILET, Ecole Nationale Vétérinaire, Maisons-Alfort); *Corynebacterium granulosum* inactivée (CG) ou Réticulostimuline: 0,5 mg par souris (aimablement fourni par le Professeur RAYNAUD, de l'Institut Pasteur); du *Bacillus* Calmette-Guérin (BCG) 'S' vivant de l'Institut Pasteur: 1 mg par souris.

A différents temps après l'injection des bactéries, la moëlle osseuse, le sang, la rate, ont été prélevés et le nombre d'UFC y a été déterminé selon la technique de TILL et MAC CULLOCH[6]. Les souris receveuses ont été irradiées léthalement au bétatron, par des électrons d'énergie 25 mev, à raison de 200 R/min, les animaux étant placés dans l'aire de l'isodose 95%. Le caractère élevé de la dose délivrée (1100 rads) s'explique en partie au moins par l'efficacité biologique relative plus basse que celle des rayons X.

Les résultats sont rapportés sur les Tableaux I, II et III. D'une façon générale, l'injection des bactéries tend à provoquer d'abord une diminution du nombre des UFC, puis une augmentation, mais cet aspect biphasique se présente différemment, selon la bactérie et l'organe considérés. On remarque en particulier, avec les deux bactéries inactivées, CG et B_{19}, que la diminution du nombre d'UFC est particulièrement sensible et rapide au niveau de la rate, puisqu'elle apparaît dès la 24ème h après l'injection. Elle est suivie, dès la 48ème h d'une augmentation qui persiste jusqu'au 15–20ème jour. Avec BCG, la chute initiale du nombre d'UFC est surtout évidente dans la moelle et le sang. Ce n'est que le 13ème jour qu'appa-

[1] J. H. HUMPHREY, Colloques du C.N.R.S. (1963), p. 401.
[2] S. I. MORSE, Nouv. Revue fr. Hémat. *8*, 541 (1968).
[3] C. STIFFEL, D. MOUTON et G. BIOZZI, Ann. Inst. Pasteur *120*, 412 (1971).
[4] R. M. FAUVE et M. B. HEVIN, Ann. Inst. Pasteur *120*, 399 (1971).
[5] L. TOUJAS, D. SABOLOVIC, L. DAZORD, Y. LE GARREC, J. P. TOUJAS, J. GUELFI et CH. PILET, Rev. Etud. clin. biol. *17*, 267 (1972).
[6] J. E. TILL et E. A. MACCULLOCH, Radiation Res. *14*, 213 (1961).